

Viraemia in patients with naturally acquired dengue infection*

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The magnitude and duration of dengue viraemia were studied in 153 patients with naturally acquired dengue infection in Jakarta, Indonesia. The duration of viraemia ranged from 2 to 12 days, but most patients had detectable circulating virus for 4-5 days. Accurate measurement of peak virus titres was not possible for many patients because of late admission to the hospital. Composite pictures of viraemia for each serotype, however, showed that many patients infected with dengue 1, 2, or 3 had circulating virus titres ranging from barely detectable to over 10^8 MID₅₀ per ml for 3-5 days. Virus titres in patients infected with dengue 4 were about 100-fold lower. Dengue haemagglutination-inhibition antibody titres of 80 or less had little effect on viraemia, but antibody titres of 160 or greater were associated with a decrease in virus isolation rate and in virus titre. The duration and magnitude of viraemia did not vary significantly with the severity of the disease and was only slightly higher in patients classified as primary dengue infections than in those classified as secondary infections. Measurement of viraemia in fatal dengue haemorrhagic fever (DHF) cases showed that these patients had significant quantities of circulating virus at the time of death.

Dengue viruses have been among the most difficult arboviruses to isolate and propagate because sensitive host systems have not been available. Accordingly, few attempts have been made to measure dengue viraemia in naturally acquired human infections. Sabin (1) estimated dengue viraemia at 10^6 minimal infective doses (MID) per ml in sera collected within a few hours of onset of disease in human volunteers. Subsequent studies on dengue fever and dengue haemorrhagic fever (DHF), however, depended on less sensitive methods, such as suckling mice and various tissue culture systems, for isolation and assay of virus (2-4). As a result, virus titres in human sera have seldom been reported.

The recent development of the mosquito inoculation technique has provided a highly sensitive

method for the isolation and propagation of dengue viruses (5). Using this technique, recent studies in Indonesia and several South Pacific islands have shown that there is considerable variation in circulating virus titres among patients infected with different strains and serotypes of dengue virus (6-9). This report describes observations made on viraemia in hospitalized DHF patients with naturally acquired infection in Jakarta, Indonesia, from 1975 to 1978.

MATERIALS AND METHODS

Most of the patients in the study were admitted to one of three sentinel hospitals located in different districts of the city. Detailed clinical observations were made on each patient and will be reported elsewhere. A detailed history was taken and an attempt was made to determine as accurately as possible the date of onset of fever and symptoms. Criteria for classification of severity of illness were as follows: (a) all patients with fever and non-specific constitutional symptoms only or with a positive tourniquet test as the only haemorrhagic manifestation were classified as dengue fever (DF);^a (b) patients who had spontaneous bleeding of some kind (purpura, ecchymoses, bleeding gums, epistaxis, haematemesis, melaena, haematuria) were

* This study was supported by funds provided by the Ministry of Health, Indonesia, and the Naval Medical Research and Development Command, Navy Department, for Work Unit MRO41.01-0151. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the Indonesian Ministry of Health, Navy Department, or the Naval Service at large. Reprint requests should be sent to: Publications Office, NAMRU-2, APO San Francisco, CA 96328, USA.

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^a This is sometimes classified as DHF, grade I.

classified as DHF, grade II; and (c) all patients with narrowing of pulse pressure (20 mmHg or less), hypotension (systolic pressure of 80 mmHg or less), or undetectable pulse and blood pressure were classified as dengue shock syndrome (DSS) (grades III and IV) (10).

Collection and processing of specimens

Venous blood samples were taken from each patient as soon as possible after admission. If patients were admitted to the hospital in the evening, finger-prick blood samples were taken immediately and a venous sample was obtained the next morning. Another venous sample (convalescent) was taken on the day of discharge from the hospital. When possible, finger-prick blood samples were taken each day until the patient became afebrile. These samples were obtained by pricking the finger with a sterile blood lancet and filling five or more plain or heparinized haematocrit capillary tubes (75 mm long) with blood. One end of each capillary tube was sealed with clay and all tubes containing blood from a single patient were placed in a covered test tube (13 × 100 mm) containing pertinent information for storage and transportation. All blood samples were stored in a refrigerator at 4 °C until they were taken to the virology laboratory.

Specimens (both venous and finger-prick) were transferred on a daily basis to the virology laboratory where the serum was separated by centrifugation and stored in a mechanical freezer at below -60 °C. Venous blood samples were handled according to standard procedure (11), but serum or plasma from finger-prick samples was separated by centrifugation for 10 minutes in a haematocrit centrifuge.

Serology and virology

Paired sera were tested for dengue antibodies by the haemagglutination-inhibition (HI) test with modification to microtitre (12). Eight units of dengue type 2 antigen were used in the initial screening. Acute and convalescent sera from the same patient were always tested together using serial 2-fold dilutions. Paired sera that did not have any detectable antibody to dengue 2 antigen in either specimen were retested using dengue 1 and dengue 3 antigens and, occasionally, dengue 4. All dengue antigens were prepared from infected mouse brains of the prototype strains (Hawaiian, New Guinea C, H87, H241) by sucrose-acetone extraction. Non-specific inhibitors were removed from the sera by treatment with acid-washed kaolin and adsorption with goose red blood cells.

Virus isolation was attempted from the first serum sample taken from all patients who showed a 4-fold or greater rise in antibody titre against any of the dengue antigens. In addition, sera or tissue biopsy specimens from all fatal cases with a possible clinical diagnosis of

dengue infection were inoculated for virus isolation (13).

All virus isolation attempts were done by the mosquito inoculation technique (5). Sera, both undiluted and at a dilution of 1:5 in phosphate-buffered saline (PBS) with 50 ml of heat-inactivated (56 °C for 30 minutes) calf serum per litre, were inoculated intrathoracically into female *Aedes aegypti* or *A. albopictus*. After 14–20 days of incubation at 32 °C, the salivary glands were dissected from surviving mosquitos and placed on a glass microscope slide along with the brain squash from the same insect. The presence of viral antigen in the brain and salivary glands was determined by the direct fluorescent antibody test (DFAT) (14). Virus isolates were identified by the complement fixation test employing antigen prepared in male mosquitos (15).

Sera from which virus was isolated were titrated by preparing serial 10-fold dilutions in PBS with 50 ml of heated calf serum per litre and inoculating each dilution into groups of uninfected male mosquitos. After incubation for 10–14 days at 32 °C, the mosquitos were killed by freezing and the presence of viral antigen in the brain was determined by DFAT. Generally, at least 5 mosquitos per dilution were tested. Virus titres were calculated by the method of Reed & Muench (16) and expressed in terms of the dose that infected 50% of the mosquitos inoculated (MID_{50} per ml).

If the first serum taken from a patient was positive for virus, subsequent samples, if available, were also tested. Depending upon the virus titre in the first serum, subsequent sera were either inoculated undiluted and at a dilution of 1:5, or titrated, beginning with the undiluted serum.

Patients were classified according to the following criteria:

(a) Infections were considered primary if the second serum taken on or after the 14th day of illness had an HI titre of 640 or less, and the acute serum obtained before the fourth day of illness had no detectable HI antibody (titre < 10).

(b) All patients having a convalescent serum HI titre of 1280 or greater and/or titre of 10 or greater in the acute serum were considered to have secondary dengue infection (17).

RESULTS

Between October 1975 and June 1978, 160 virologically confirmed dengue patients were studied in Jakarta and of these, adequate clinical data were available for 153. The majority of patients (51%) were in the 5–9 year age group, with 25% and 17% in the 0–4 and 10–14 year age groups, respectively. There

were more males (54%) than females (42%), and the sex of 6 patients (4%) was not recorded.

Viraemia was assumed to have started on the day of onset of fever, as given on admission to the hospital, and to have ended on the last day of illness that virus was detected in the blood by the mosquito inoculation

Table 1. Duration of viraemia in hospitalized dengue patients in Jakarta, Indonesia

Duration of viraemia ^a (days)	No. of patients				Total
	Dengue 1	Dengue 2	Dengue 3	Dengue 4	
1	—	—	—	—	—
2	1	3	7	—	11
3	1	12	15	1	29
4	6	12	22	4	44
5	9	6	15	1	31
6	7	5	4	—	16
>7	4	5	5	3	17
Unknown	—	1	4	—	5
Total	28	44	72	9	153
Mean duration of viraemia ^b	5.1 ± 0.2	4.7 ± 0.3	4.1 ± 0.2	5.0 ± 0.5	4.5

^a Estimated from day of onset reported on admission to hospital.

^b In days ± SE.

technique. Table 1 shows the minimum duration of dengue viraemia for the four virus serotypes in 153 patients. The duration of viraemia varied from 2 to 12 days, but the majority of patients had circulating virus for 4–5 days. The median duration of viraemia for dengue 2, 3, and 4 was 4 days, whereas the median for dengue 1 was 5 days. The mean duration of viraemia was 5 days in patients infected with dengue 1, 2, and 4 and 4 days in patients infected with dengue 3.

A composite picture of the magnitude of viraemia for each dengue serotype by day of illness is shown in Table 2. Patients infected with dengue 1 had virus titres ranging from 3.8 (barely detectable) to 8.0 log₁₀MID₅₀ per ml. Ranges and geometric mean titres showed little change up to day 6 of the illness. Four patients were admitted to hospital on day 7 of their illness, or later. All had viraemia on admittance but the virus titres were barely detectable.

Dengue 2 virus titres were somewhat higher, ranging from 3.8 to over 8.3 log₁₀MID₅₀ per ml up to day 6 of infection. Geometric mean virus titres for this serotype were also slightly higher than those for dengue 1. The longest viraemia recorded for dengue 2 was 12 days, and there were six patients with detectable viraemia for 7 days or longer. Four of these had viraemia for 10 days or longer but, as with dengue 1, virus titres were low.

Dengue 3 virus titres were similar to those for dengue 2, ranging from 3.8 to over 8.3 log₁₀MID₅₀ per ml up to day 4 of illness, after which they began to drop. Geometric mean virus titres, however, were similar to those for dengue 1. Five patients had viraemia lasting 7 days or longer, the longest being 8 days.

Table 2. Geometric mean virus titres in sera from hospitalized patients with dengue infection by day of illness

Day of illness ^a	Dengue 1		Dengue 2		Dengue 3		Dengue 4	
	No. of sera	Geometric mean titre ^b	No. of sera	Geometric mean titre ^b	No. of sera	Geometric mean titre ^b	No. of sera	Geometric mean titre ^b
1	—	—	—	—	—	—	—	—
2	1	7.3	3	6.3 ± 1.2	8	5.3 ± 0.5	—	—
3	1	4.0	12	5.7 ± 0.4	16	5.1 ± 0.3	2	6.0 ± 0.2
4	7	4.5 ± 0.3	12	5.1 ± 0.4	23	4.8 ± 0.3	4	4.9 ± 0.5
5	10	4.9 ± 0.4	7	5.0 ± 0.8	15	4.4 ± 0.2	1	6.0
6	8	4.5 ± 0.3	6	5.2 ± 0.8	4	4.0 ± 0.1	—	—
>7	4	3.8	6	4.0 ± 0.1	5	4.8 ± 0.5	3	4.8 ± 0.6
Unknown	—	—	1	6.3	4	3.9 ± 0.1	—	—
Total	31	4.6 ± 0.2	47	5.2 ± 0.2	75	4.8 ± 0.1	10	5.2 ± 0.3

^a Estimated from day of onset given on admission to hospital.

^b log₁₀MID₅₀ per ml ± SE.

Table 3. Relationship between HI antibody titre and dengue virus isolation rate from human sera

HI antibody titre	No. of positive sera/ no. tested	%
<10	45/80	56.2
10	15/31	48.4
20	20/29	69.0
40	18/27	66.7
80	19/44	43.2
160	25/70	35.7
320	7/75	9.3
640	3/71	4.2
1280	1/13	7.7
Total	153/440	34.8

Only 10 sera were titrated for dengue 4, giving virus titres ranging up to 6.3 log₁₀MID₅₀ per ml. Three patients had detectable viraemia up to day 7 of the illness.

The influence of dengue HI antibody titre on viraemia is shown in Tables 3 and 4. It will be noted that HI antibody titres of 80 or less had little effect on viraemia as indicated by the virus isolation rate (Table 3) and by virus titres (Table 4). Both parameters decreased in patients with acute serum HI titres of 160 or greater.

The relationship between duration of viraemia and severity of disease is shown in Table 5. The data showed no apparent relationship between minimum duration of viraemia and severity of disease for any of the serotypes. For patients with dengue 1, the longest mean duration of viraemia was associated with grade II illness; for dengue 2 and 4 patients, with grade III

Table 4. Comparison of dengue virus titre and HI antibody titre in human sera

HI antibody titre	Dengue 1		Dengue 2		Dengue 3		Dengue 4		Total	
	No. of sera	Virus titre ^a	No. of sera	Virus titre ^a	No. of sera	Virus titre ^a	No. of sera	Virus titre ^a	No. of sera	Virus titre ^a
<10	14	5.1	11	5.9	20	5.0	—	—	45	5.3
10	2	4.2	5	6.5	6	5.5	2	6.1	15	5.7
20	3	4.9	5	5.4	10	5.0	2	5.2	20	5.1
40	3	4.5	5	4.8	10	4.5	—	—	18	4.6
80	1	3.8	8	5.1	9	4.5	1	6.0	19	4.8
160	3	3.9	9	4.0	12	3.9	1	3.8	25	3.9
320	1	3.8	—	—	5	3.9	1	5.3	7	4.1
640	1	4.3	—	—	—	—	2	4.2	3	4.2
1280	—	—	1	4.3	—	—	—	—	1	4.3

^a Geometric mean virus titre, log₁₀ MID₅₀ per ml

Table 5. Duration of viraemia in hospitalized patients with dengue infection by virus serotype and severity of disease

Disease severity	Dengue 1		Dengue 2		Dengue 3		Dengue 4	
	No. of patients	Mean duration of viraemia ^a	No. of patients	Mean duration of viraemia ^a	No. of patients	Mean duration of viraemia ^a	No. of patients	Mean duration of viraemia ^a
DF	7	4.9 ± 0.5	8	3.9 ± 0.5	16	4.1 ± 0.2	4	5.0 ± 0.7
Grade II	7	5.7 ± 0.5	10	3.9 ± 0.4	8	4.6 ± 0.6	2	5.0 ± 2.0
Grade III	10	5.1 ± 0.3	17	5.9 ± 0.8	18	4.7 ± 0.3	2	5.5 ± 1.5
Grade IV	4	4.8 ± 0.9	9	4.0 ± 0.3	27	3.7 ± 0.2	1	4.0 —
Total	28	5.1 ± 0.2	44	4.7 ± 0.3	69	4.1 ± 0.2	9	5.0 ± 0.5

^a In days ± SE.

Table 6. Geometric mean dengue virus titres in sera from hospitalized patients by virus serotype and severity of disease

Disease severity	Dengue 1		Dengue 2		Dengue 3		Dengue 4	
	No. of patients	Geometric mean titre ^a	No. of patients	Geometric mean titre ^a	No. of patients	Geometric mean titre ^a	No. of patients	Geometric mean titre ^a
DF	7	4.3 ± 0.2	8	5.7 ± 0.6	16	4.7 ± 0.3	4	4.9 ± 0.5
Grade II	7	5.2 ± 0.6	10	4.8 ± 0.3	8	4.9 ± 0.5	2	5.2 ± 0.6
Grade III	10	4.5 ± 0.3	17	5.3 ± 0.4	18	4.8 ± 0.3	2	6.1 ± 0.1
Grade IV	4	4.7 ± 0.8	9	5.1 ± 0.6	27	4.7 ± 0.3	1	3.8
Total	28	4.7 ± 0.2	44	5.2 ± 0.2	69	4.7 ± 0.1	9	5.1 ± 0.3

^a Log₁₀ MID₅₀ per ml ± SE.

illness; and for dengue 3 patients, with grade II and grade III illness.

Similarly, geometric mean dengue virus titres showed little consistent variation with severity of disease, or among patients with equally severe infections with different dengue serotypes (Table 6).

The relationship between dengue 1, 2, and 3 viraemia and severity of disease was analysed in patients classified as having primary and secondary infections, but there was no apparent variation in virus titres with grade of illness. However, in patients infected with dengue 1 and 3, the geometric mean virus titres were about 5-fold higher in primary than in secondary infections (Table 7). In patients infected with dengue 2, virus titres were the same in primary and secondary infections. All patients infected with dengue 4 had secondary infections.

The mean duration of viraemia in primary and

secondary infections is also shown in Table 7. The minimum duration of viraemia was slightly shorter in patients experiencing secondary infections with dengue types 1 and 2. With dengue 3, however, patients with secondary infections had circulating virus for about half a day longer than those with primary infections.

During the course of this study, dengue viruses were isolated from 30 fatal cases. Table 8 presents viraemia data for these dengue patients by day of illness and serotype. There were three fatal dengue 1 infections, five dengue 2, 21 dengue 3, and one dengue 4. Viraemias ranged from barely detectable (3.8) to over 7.3 log₁₀ MID₅₀ per ml. Moreover, virus was isolated from the liver of one dengue 1 patient with a virus titre of 5.0 log₁₀ MID₅₀ per ml in that tissue. There were no apparent differences among the serotypes with regard to duration or magnitude of viraemia in fatal DHF.

Table 7. Mean duration and magnitude of viraemia in hospitalized patients with primary and secondary dengue infections

Dengue serotype	Primary infections			Secondary infections		
	No. of cases	Mean duration of viraemia ^a	Geometric mean titre ^b	No. of cases	Mean duration of viraemia ^a	Geometric mean titre ^b
Dengue 1	12	5.5 ± 0.3	5.0 ± 0.4	16	4.9 ± 0.3	4.4 ± 0.2
Dengue 2	11	5.4 ± 0.9	5.3 ± 0.5	32	4.3 ± 0.3	5.2 ± 0.3
Dengue 3	19	3.8 ± 0.2	5.2 ± 0.3	49	4.3 ± 0.2	4.5 ± 0.1
Dengue 4	—	—	—	9	5.0 ± 0.5	5.1 ± 0.3

^a In days ± SE.

^b Log₁₀ MID₅₀ per ml ± SE.

Table 8. Dengue virus titres in sera from patients with fatal dengue infection by day of illness and virus serotype

Day of illness ^a	Dengue 1		Dengue 2		Dengue 3		Dengue 4	
	No. of patients	Geometric mean titre ^b	No. of patients	Geometric mean titre ^b	No. of patients	Geometric mean titre ^b	No. of patients	Geometric mean titre ^b
1	—	—	—	—	—	—	—	—
2	1	7.3	—	—	3	4.7	—	—
3	—	—	3	5.2	6	4.5	—	—
4	—	—	1	5.3	6	5.3	1	3.8
5	1	3.8	1	3.8	3	4.5	—	—
6	1	4.0	—	—	1	3.8	—	—
7	—	—	—	—	1	6.3	—	—
Unknown	—	—	—	—	1	3.8	—	—
Total	3	5.0	5	4.9	21	4.8	1	3.8

^a In most cases, only one blood sample was taken from each patient, i.e., on the day of admission.

^b Log₁₀ MID₅₀ per ml.

DISCUSSION

Viraemia is an important aspect of dengue infection, but has not been extensively investigated. It is important because the amount of circulating virus in patients, along with the competence and density of the mosquito vector population, has a significant influence upon the transmission dynamics of the virus. Furthermore, the level of viraemia in a patient may be related to the severity of the illness (6, 9).

Data on viraemia in naturally acquired human dengue infections are very limited. In addition to Sabin's original titrations using human volunteers (1), only five recorded attempts have been made to measure the magnitude and duration of dengue viraemia in man (5–9). All of these studies dealt with epidemic dengue and showed that there is considerable variation in the amount of circulating dengue virus among different serotypes as well as among different strains of the same serotype. Other workers have not specifically titrated positive human sera to measure virus content. This has been done, however, in experiments with rhesus monkeys (18), cynomolgus monkeys (L. Rosen & D. J. Gubler, unpublished data, 1974), and chimpanzees (19). With all these species, virus titres were generally lower than those observed in human subjects in this study.

It is difficult to obtain accurate data on the maximum virus titres in hospitalized DHF patients, because they are usually admitted long after the onset of infection, when the level of viraemia may already be decreasing. Therefore, our measurements of virus titre probably underestimated peak viraemia in most

of the patients in this study. Nevertheless, many children had significant quantities of circulating dengue virus as long as 6–7 days after onset. Geometric mean virus titres were between 10⁴ and 10⁵ MID₅₀ per ml for all four serotypes until day 6 of illness. The mean minimum duration of viraemia was also similar for the four dengue viruses, being shortest in patients infected with dengue 3 (4.1 days) and longest in those with dengue 1 (5.1 days). The overall mean minimum duration for 153 patients was 4.5 days. A histogram

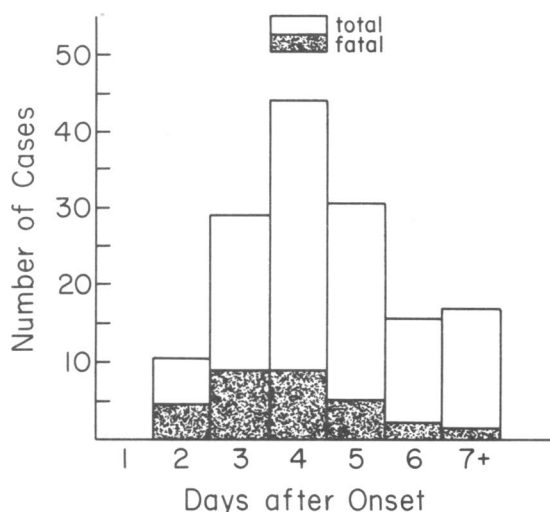


Fig. 1. Histogram showing duration of viraemia in hospitalized dengue patients, Jakarta, Indonesia.

showing duration of viraemia in all patients, including those with fatal infections, is presented in Fig. 1. The median duration of viraemia was 4 days for all cases and 3.5 days for fatal cases. The latter is probably an underestimate, since most specimens from which virus was isolated were taken before death.

The relationship between dengue HI antibody and dengue viraemia has not been widely studied. In an earlier report, it was found that most dengue virus isolations were made from sera with dengue HI antibody titres of 80 or less (8). This is in close agreement with the present study where it was found that isolation rates and virus titres began to decrease in sera with dengue HI antibody titres of 160 or greater.

Data on the duration and magnitude of viraemia in relation to severity of disease and virus serotype were presented in Tables 5 and 6. When patients infected with each serotype were examined separately, no marked differences in either duration or magnitude of viraemia were observed between patients with mild and severe disease.

It has been proposed that dengue virus replication in man occurs mainly in mononuclear phagocytes and that the pathogenesis of DHF may be related to the number of these cells infected with dengue virus (20). Thus, those patients with severe and fatal disease should have more infected mononuclear cells and presumably more virus. This would imply that patients with more severe disease should have higher viraemias than those with milder disease, provided virus is released from the cells. Our data do not

support this conclusion. There was no difference in the amount of circulating virus in mild and severe disease. However, patients experiencing primary dengue 1 or 3 infection had geometric mean virus titres about 5-fold higher than patients with secondary infection with the same serotype. This difference was not observed in patients with dengue 2 infection. Studies with rhesus monkeys have shown similar differences (18). Viraemias were higher in monkeys with primary dengue 1 or 4 infection than in those with secondary infection. In monkeys infected with dengue 2, however, viraemias were 13-fold higher in animals with secondary infections.

It has been reported that the blood of patients with severe dengue shock syndrome may be virologically sterile because of the antigen-antibody complexing that occurs (21). It has been proposed that this may explain the lack of virus isolations from fatal DHF cases. It will be noted from Table 8 that this is obviously not the case with Jakarta DHF patients. In all, 33 fatal cases of dengue infection were virologically confirmed over a 2½-year period. Of these, 70% were associated with dengue 3 infections (22), but fatalities were associated with all four dengue serotypes. Virus titres in some fatal dengue infections in Jakarta were over 10^8 MID₅₀, although the majority of patients had lower titres. Our success in isolating dengue viruses from fatal cases is most probably due to the use of the mosquito inoculation technique, which is more sensitive than methods used previously.

ACKNOWLEDGEMENTS

We thank Ms Chairin Maroef, Woro Djuarti, Masran Masri, and Hardianto Saipan for excellent technical assistance, Dr N. J. Marchette, University of Hawaii, for kindly providing the hyperimmune mouse ascitic fluid used for typing the viruses, and Dr T. Ksiazek, NAMRU-2, Taipei, for providing viral antigens for HI. We are also grateful to Dr Julie Sulianti Saroso, previous Director, and Dr A. A. Loedin, present Director, National Institute of Health Research and Development, Ministry of Health, Indonesia, and to Dr Adhyatma, Director General, Communicable Disease Control, Ministry of Health, Indonesia, for making this study possible.

RÉSUMÉ

LA VIRÉMIE CHEZ LES MALADES SOUFFRANT DE DENGUE NATURELLEMENT CONTRACTÉE

Les virus de la dengue figurent parmi les arbovirus les plus difficiles à isoler et à cultiver en raison de l'absence de systèmes hôtes sensibles. La mise au point récente de la technique d'inoculation au moustique a toutefois permis de résoudre ce problème. Le présent rapport décrit les observations faites sur la virémie chez des malades hospitalisés pour dengue naturellement contractée à Djakarta, Indonésie, d'octobre 1975 à juin 1978.

Des échantillons de sang étaient quotidiennement prélevés

et expédiés au laboratoire, et le sérum était conservé à une température de -60°C ou plus basse. Tous les isolements de virus et tous les titrages ont été réalisés par la technique d'inoculation au moustique et par immunofluorescence directe.

L'intensité et la durée de la virémie ont été étudiées chez 153 malades atteints de dengue. La plupart d'entre eux (51%) appartenaient au groupe d'âge 5-9 ans, avec à peu près autant de filles que de garçons. La durée minimale de la

virémie était de 2 à 12 jours, mais chez la plupart des malades on détectait des virus circulants pendant 4 à 5 jours. On n'a observé aucune différence apparente en ce qui concerne la durée de la virémie entre les quatre sérotypes de la dengue. Chez de nombreux malades, il n'a pas été possible de mesurer exactement les titres maximaux de virus en raison de leur hospitalisation tardive. Toutefois, l'examen des valeurs de la virémie pour chaque sérotype a montré que de nombreux malades infectés par les sérotypes 1, 2 et 3 avaient des titres de virus circulants échelonnés d'à peine décelables à plus de 10^6 DIM₅₀ par ml, pendant 3 à 5 jours. Les titres de virus chez les malades infectés par la dengue de type 4 étaient

environ 100 fois plus faibles. Des titres d'anticorps IH de 80 ou moins avaient peu d'effet sur la virémie, tandis que des titres de 160 ou plus étaient associés à une baisse du taux d'isolement des virus et des titres de virus. La durée et l'intensité de la virémie ne différaient pas sensiblement dans les maladies graves et les formes bénignes, et la virémie n'était pas beaucoup plus élevée chez les malades atteints d'une infection primaire que chez ceux atteints d'une infection secondaire. La mesure de la virémie dans les cas mortels de dengue hémorragique a montré qu'au moment du décès les malades hébergeaient une quantité importante de virus circulants.

REFERENCES

1. SABIN, A. B. Research on dengue during World War II. *American journal of tropical medicine and hygiene*, 1: 30-50 (1952).
2. HAMMON, W. McD. ET AL. Viruses associated with epidemic dengue hemorrhagic fevers of the Philippines and Thailand. *Science*, 131: 1102-1103 (1960).
3. SUKHAVACHANA, P. ET AL. Tissue culture techniques for the study of dengue viruses. *Bulletin of the World Health Organization*, 35: 65-66 (1966).
4. YUILL, T. M. ET AL. Dengue virus recovery by direct and delayed plaques in LLC-MK₂ cells. *American journal of tropical medicine and hygiene*, 17: 441-448 (1968).
5. ROSEN, L. & GUBLER, D. J. The use of mosquitoes to detect and propagate dengue virus. *American journal of tropical medicine and hygiene*, 23: 1153-1160 (1974).
6. GUBLER, D. J. ET AL. Epidemiologic, clinical, and virologic observations on dengue in the Kingdom of Tonga. *American journal of tropical medicine and hygiene*, 27: 581-589 (1978).
7. KUBERSKI, T. ET AL. Clinical and laboratory observations on patients with primary and secondary dengue type 1 infections with hemorrhagic manifestations in Fiji. *American journal of tropical medicine and hygiene*, 26: 775-783 (1977).
8. GUBLER, D. J. ET AL. Epidemic dengue hemorrhagic fever in rural Indonesia. I. Virological and epidemiological studies. *American journal of tropical medicine and hygiene*, 28: 701-710 (1979).
9. GUBLER, D. J. ET AL. Epidemic dengue 3 in Central Java associated with low viremia in man. *American journal of tropical medicine and hygiene*, (in press) 1981.
10. Technical Advisory Committee on Dengue Hemorrhagic Fever for the South-East Asian and Western Pacific Regions. *Technical guides for diagnosis, treatment, surveillance, prevention and control of dengue haemorrhagic fever*, Geneva, World Health Organization, 1975.
11. LENNETTE, E. H. General principles underlying laboratory diagnosis of viral and rickettsial infections. In: Lennette, E. H. & Schmidt, N. J., *Diagnostic procedures for viral and rickettsial infections*, 4th ed., New York, American Public Health Association, 1969, pp. 1-65.
12. CLARKE, D. H. & CASALS, J. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. *American journal of tropical medicine and hygiene*, 7: 561-573 (1958).
13. SUHARYONO, W. ET AL. Dengue virus isolations in Indonesia 1975-1978. *Asian journal of infectious diseases*, 3: 27-32 (1979).
14. KUBERSKI, T. T. & ROSEN, L. A simple technique for the detection of dengue antigen in mosquitoes by immunofluorescence. *American journal of tropical medicine and hygiene*, 26: 533-537 (1977).
15. KUBERSKI, T. T. & ROSEN, L. Identification of dengue viruses using complement fixing antigen produced in mosquitoes. *American journal of tropical medicine and hygiene*, 27: 538-543 (1977).
16. REED, L. J. & MUENCH, H. A simple method of estimating fifty percent endpoints. *American journal of hygiene*, 27: 493-497 (1938).
17. RUSSELL, P. K. ET AL. Antibody response in dengue and dengue hemorrhagic fever. *Japanese journal of medical science and biology*, 20: 103-108 (1967).
18. HALSTEAD, S. B. ET AL. Studies on the pathogenesis of dengue infection in monkeys. II. Clinical laboratory responses to heterologous infection. *Journal of infectious diseases*, 128: 15-22 (1973).
19. SCHERER, W. F. ET AL. Experimental infection of chimpanzees with dengue viruses. *American journal of tropical medicine and hygiene*, 27: 590-599 (1978).
20. HALSTEAD, S. B. ET AL. Immunologically enhanced dengue virus infection of mononuclear phagocytes. A mechanism which may regulate disease severity. *Asian journal of infectious diseases*, 2: 85-93 (1978).
21. HALSTEAD, S. B. Observations related to pathogenesis of dengue hemorrhagic fever. VI. Hypothesis and discussion. *Yale journal of biology and medicine*, 42: 350-362 (1970).
22. GUBLER, D. J. ET AL. Virological surveillance for dengue haemorrhagic fever in Indonesia using the mosquito inoculation technique. *Bulletin of the World Health Organization*, 57: 931-936 (1979).